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Analytical applications of pH-ISFETs

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Abstract

This work presents a brief review of the results obtained in the Chemical Sensors Laboratory at the University of St. Petersburg and gives some examples of possible applications of pH-sensitive field-effect transistors with zirconium dioxide and tantalum pentoxide gate films. Specifically, the following applications are discussed: (i) direct pH measurements in blood and gastric juice; (ii) pH-ISFETs as a base for enzyme sensors (urea, butyrylcholine); (iii) the determination of inhibitors with an enzyme sensor; (iv) application of pH-ISFETs for direct pH measurements in microbiological systems.

1. Introduction

Intensive investigations of pH-sensitive field-effect transistors during the last few years [1, 2] have led to the fabrication of sufficiently small, rugged, convenient to use and stable pH sensors that are now commercially available. Further development in this field undoubtedly requires enhanced efforts aimed at the practical application of these sensors in medicine, biology, agriculture, environmental studies, etc. The aim of this work is to show particular examples of the practical application of pH-ISFETs.

2. ISFET fabrication

In this work ion-sensitive field-effect transistors (ISFETs) of different designs were used and were fabricated on p-type (10–40 Ω cm) silicon wafers of ⟨100⟩ orientation. The ISFET's channel length was 20 μm and the width varied in the range 0.5–2 mm to give devices with various transconductance values. The gate region was formed by a thermally grown SiO_2 layer (70–100 nm) and a pH-sensitive film (ZrO_2 or Ta_2O_5). The fabrication processes of these devices are presented in more detail elsewhere [3, 4]. All sensors were made using standard planar technology and epoxy resin was used for encapsulation. The processes of ISFET gate formation were optimized to obtain threshold voltage values lying in the range −2.5 to −1.5 V in solutions with pH = 7. This guarantees that small gate voltages would be applied to the device under working conditions.

A special microprocessor-based pH-meter was designed for use with the ISFET probes. The analog circuit of the pH-meter sustains a constant value of the ISFET drain current in the range 0.2–1.5 mA under a constant applied source-to-drain voltage (0.5–5 V). The output voltage, V_G, is related to a solution pH value according to

$$V_G = V_0 - S\text{pH} \quad (1)$$

where V_0 is an ISFET constant and S is the chemical sensitivity (mV/pH). Since the ISFETs transconductance is higher than 1 mA/V, the output voltage can be measured with a precision of 0.1 mV, corresponding to 0.002 pH units. Both coefficients in eqn. (1) are determined by sensor calibration in two buffer solutions and are stored in the pH-meter memory. For precise measurements, the ISFET's long-term drift and temperature sensitivity must be compensated. If the corresponding coefficients for the sensor are known, the resulting pH value can be corrected with the help of a microprocessor.

3. Characteristics of pH-ISFETs

pH-ISFETs with ZrO_2 and Ta_2O_5 gate films showed a linear pH dependence in the pH range 1–12 [3, 4], with sensitivities of 56 ± 0.3 and 57 ± 1 mV/pH, respectively. It was shown [4, 5] that the pH sensitivity is independent of the solution ionic strength in the range 10^{-3}–1 mol/l. The selectivity coefficients towards alkaline ions were found to be comparable with those of a conventional glass electrode. The response time of pH-ISFETs measured in a flow-through cell was < 1 s. A larger response time was found for ZrO_2-ISFETs in solutions with a high (> 10^{-1} mol/l) sodium ion concentration. Other alkaline ions do not alter this parame-

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ter. The long-term drift of the sensor's output voltage did not exceed 0.5–1 mV/h during eight hours of measurements in solutions with constant pH.

4. pH-ISFETs in medical investigations

4.1. pH measurements in blood

The most promising field of ISFET applications is medicine and biology. However, the complex composition of biological fluids requires a thorough investigation of possible interference from the analyte components. Earlier it was shown [6, 7] that pH-ISFETs with Si_3N_4 and Al_2O_3 gate films can be used for continuous monitoring of the whole blood pH. In this work the characteristics of ZrO_2- and Ta_2O_5-ISFETs were investigated in plasma, whole blood and blood enriched with blood cells (erythrocytes, etc.).

Figure 1 presents the ZrO_2-ISFET response obtained in heparinized whole blood titrated with small amounts of HCl or KOH (0.1 mol/l) versus the pH value of the sample measured by a glass pH-electrode. Over the physiologically meaningful pH range the sensor shows a linear pH response. It must be noted that to stabilize the sensor's characteristics after first contact with blood samples, it must be presoaked in the sample for 15–20 min. The response time to rapid pH changes in blood was a little higher than in standard buffer solutions ($\tau_{98\%} \approx 1$ min). This can be attributed to the high (0.1 mol/l) sodium ion concentration in blood. The stability of the sensor characteristics during its contact with blood was controlled by testing its output voltage in a standard buffer. After five hours of measurements, fluctuations did not exceed ±1 mV. pH measurements

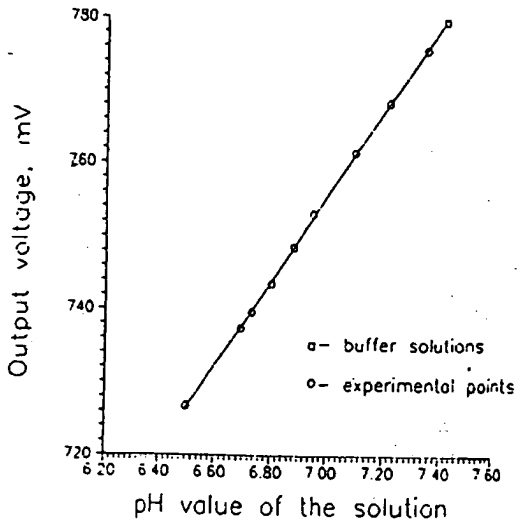

Fig. 1. pH response of ZrO_2-ISFET in whole heparinized blood titrated with small amounts of NaOH and HCl.

TABLE 1. Direct pH measurements made in whole blood with ZrO_2-gate ISFET

Glass electrode	6.75	7.36	7.24	7.10	6.95	6.75	6.51
ISFET	6.73	7.37	7.23	7.09	6.93	6.74	6.55

made in plasma, whole blood and blood enriched with blood cells showed no visible differences in sensor behaviour, although prolonged experiments in these fluids were not done. The experimental data obtained are presented in Table 1. pH values measured with ISFETs were calculated from two calibration points (pH 6.88 and pH 7.42).

pH-ISFETs with Ta_2O_5 gate films also showed a linear pH response in blood samples, but their response time was much higher (2–5 min) compared with ZrO_2-ISFETs. It is supposed that the large response-time values in this case are due to the formation of an adsorbed protein layer that has its own buffer capacity. It was found that contact with blood results in a shift of the ISFET's calibration curve along the voltage axis. A correlation between the shift and the blood-cell content in the sample was established [8]. While in plasma the difference between the pH measured with a glass electrode and with a Ta_2O_5-ISFET was 0.05–0.1 units, in blood enriched with blood cells the error was 0.3–0.4 pH. In buffer solution the sensor output value returned to its initial value after 5–10 min. These effects are probably associated with adsorption of proteins at the Ta_2O_5-ISFET gate.

4.2. pH-ISFETs in gastroenterological investigations

The quantitative determination of hyperacidity or hypoacidity in the stomach is of great importance for the diagnosis of various diseases. Usually special probes with miniature glass pH-electrodes or antimony/antimony oxide electrodes are used [9] for *in vivo* pH measurements in gastroenterological investigations. The former suffer from a high output impedance; the latter have a non-linear pH response and can easily be contaminated by various organic compounds. Preliminary experiments [10] showed that ISFETs can successfully be used for this purpose. The experimental data obtained from comparative pH measurements of gastric juices made *in vitro* with a glass electrode, antimony electrode and with ZrO_2- and Ta_2O_5-ISFETs are summarized in Table 2.

5. Enzyme sensors based on pH-sensitive ISFETs

ISFETs are widely used for constructing enzyme sensors (EnFETs) by coupling them with a membrane containing an immobilized enzyme [11–13]. If an

TABLE 2. Comparative pH measurements in gastric juice

Glass electrode	4.49	1.12	3.28	4.12	2.02	1.20	1.82
Antimony electrode	4.34	1.98	3.40	4.20	2.18	1.80	2.01
ZrO_2-ISFET	4.48	1.10	3.21	4.13	2.04	1.22	1.81
Ta_2O_5-ISFET	4.50	1.13	3.24	4.09	2.03	1.22	1.84

enzyme-catalysed reaction leads to the formation of acidic or alkaline products, then a pH-ISFET can be used to measure the change in pH and thereby to determine the activity of a substrate.

5.1. Urea sensor

Usually an enzyme-containing membrane is deposited directly on the gate region of the pH-ISFET. We tried to use another approach [14] in which the membrane is placed in a special holder mechanically attached to the gate region of the sensor. In this case the membrane can be easily changed after a decrease of enzyme activity. As an example, a ZrO_2-ISFET and an albumin membrane with immobilized urease were used. The measurements of urea concentration were carried out in phosphate buffer (10 mmol/l, pH 7.4) solutions. The response time of the urea sensor (Fig. 2) was about 2 min and the calibration plot (Fig. 3) was linear in the concentration range 0.02–1.0 mmol/l urea. The sensor response remained stable (± 5 mV) during all the experiments (10 days).

5.2. Inhibitors determination by butyrylcholine sensor

Up to now enzyme field-effect transistors (EnFETs) have mostly been used [11–13] to determine substrate concentration (glucose, urea, penicillin), but these analytical devices can also be utilized to determine inhibitors (compounds that cause a decrease in the rate of

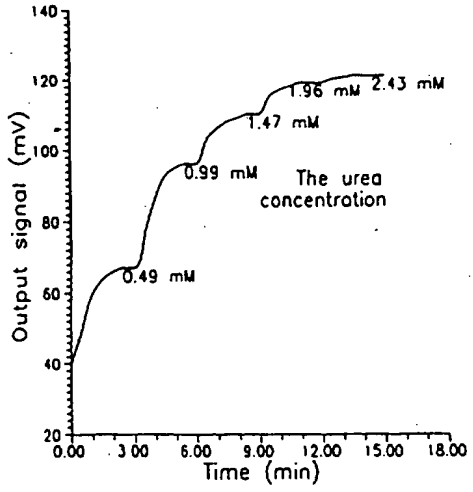

Fig. 2. Response time of urea EnFET sensor.

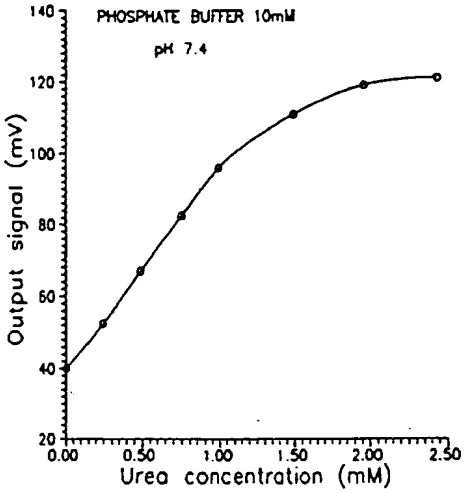

Fig. 3. The chemical response of the urea EnFET sensor.

enzyme reaction), by reacting with the enzyme or enzyme–substrate intermediate to form a corresponding complex [15]. Cholinesterase derivatives are widely used to detect various inhibitors, including such toxic compounds as pesticides [15–17]. Results on an ISFET sensitive to acetylcholine have been reported [18]. The characteristics of butyrylcholinesterase-based EnFETs have been described [19], where the possibility of sensor application for the detection of inhibitors was shown.

Butyrylcholinesterase (BCE) catalyses the reaction

$$\text{Butyrylcholine} \xrightarrow{\text{BCE}} \text{choline} + \text{butyric acid} \qquad (2)$$

Reaction (2) produces butyric acid and thus changes the pH value in the membrane. An EnFET membrane was formed from a gelatine solution, prepared on phosphate buffer (0.01 mol/l, pH 7.5), containing butyrylcholinesterase on the Ta_2O_5-ISFET gate. Optimal parameters of the membrane formation process, the pH value and buffer capacity of the working solution were presented in ref. 19.

Figure 4 shows the EnFET calibration curves in solutions of butyrylcholine iodide with different phosphate buffer concentrations.

Inhibition can be characterized according to its influence on enzyme catalytic activity as reversible or irreversible. In contrast to irreversible inhibition, the effects of reversible inhibition on the enzyme sensor characteristics can be eliminated by soaking the sensor in substrate solution. Analytical working curves for inhibitor determination are generally constructed by plotting percentage inhibition (i%) versus concentration of inhibitor [15]. The percentage inhibition is calculated according to

$$i\% = \left(\frac{V_{inh} - V_{sub}}{V_0 - V_{sub}}\right) \times 100 \qquad (3)$$

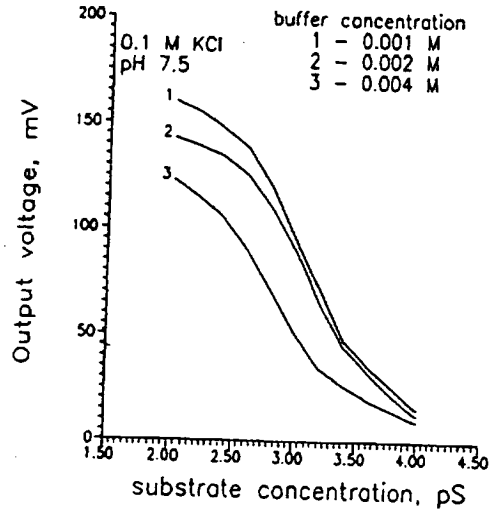

Fig. 4. Calibration curves of butyrylcholine sensor in solutions with different buffer concentrations (pS = negative logarithm of substrate concentration).

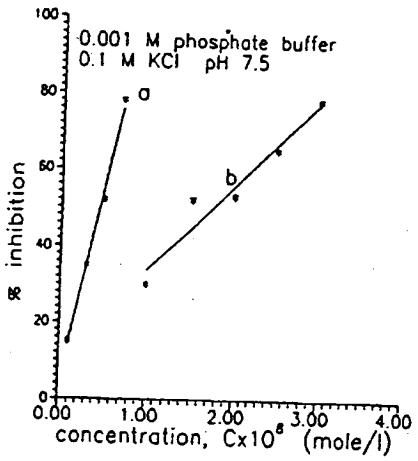

Fig. 5. Reversible inhibition of butyrylcholine sensor characteristics by tacrin. Substrate concentration 2×10^{-4} mol/l (a) and 5×10^{-4} mol/l (b).

where V_0 is the sensor output voltage in a buffer solution, V_{sub} is the sensor output voltage in a buffer solution containing substrate and V_{inh} is the sensor output voltage in a buffer solution containing substrate and inhibitor (in the case of reversible inhibition) or in a buffer solution containing substrate (for irreversible inhibition). Figure 5 shows the sensor response to tacrin (a drug that is used in medicine), which acts in this case as a reversible inhibitor, in solutions with different substrate concentrations. The plots presented reveal that the detection limit of this method depends on the substrate concentration in the working solution. The lowest detection limit in the case of tacrin is 10^{-9} mol/l.

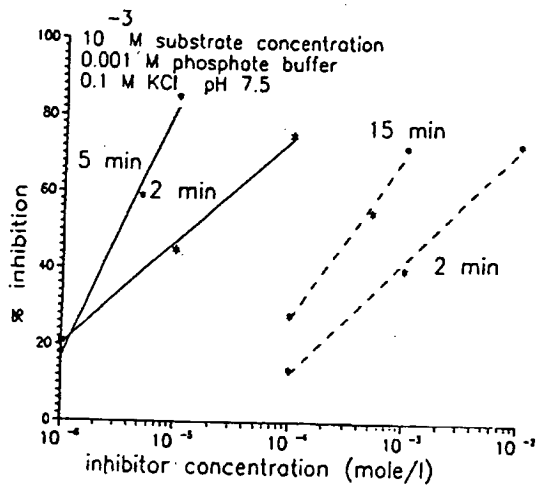

Fig. 6. EnFET sensitivity to the irreversible inhibitors DFP (solid line) and DDVP (dashed line). Time of sensor contact with a sample solution is a parameter.

The influence of irreversible inhibitors on EnFET parameters was studied using two pesticides, dimethyldichloridevinylphosphate (DDVP) and diisopropylfluorphosphate (DFP). The EnFET characteristics were measured in substrate solution (10^{-3} M), then it was immersed in a solution containing a specific amount of inhibitor for a strictly determined period of time (2–15 min). After that the sensor was rinsed and its characteristics in the substrate solution were measured once more. The percentage inhibition was calculated according to eqn. (3). Experimental data are shown in Fig. 6. Each point on the plot presents the result obtained with an EnFET with a newly made membrane. The output voltage of different sensors was reproducible with an accuracy of 10%. If the percentage inhibition is lower than 30–40%, the EnFET can be used more than once. Detection limits of 5×10^{-5} M for DFP and 10^{-6} M for DDVP were observed. It must be noted that lower concentrations can be detected using other analytical methods based on enzyme inhibition in a homogeneous phase [16]. This difference can be explained by the fact that immobilized enzyme is much less susceptible to inhibitors due to the protection of the immobilization matrix [17].

6. Application of pH-ISFETs for microbiological investigations

A pH-sensitive ISFET with a Ta_2O_5 gate film has been used for continuous pH measurements in small (0.2 ml) volumes of solutions of six different substrates containing the microorganism *Pseudomonas aeruginosa* or *Staphylococcus aureus* [20]. All measurements have

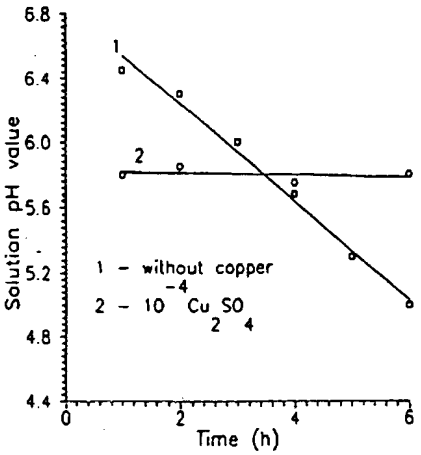

Fig. 7. The kinetics of pH changes measured with Ta_2O_5-ISFET in glucose solution containing *Staphylococcus aureus* with (2) and without (1) addition of Cu_2SO_4 (10^{-4} mol/l).

been carried out with the help of a specially designed microprocessor-based pH-meter. The sensor used provided an accuracy of 0.01 pH units.

The kinetics of pH changes in the solutions under investigation have been determined for a six-hour interval. As shown in Fig. 7, it has been found that in solutions of some substrates the pH–time dependence is nearly linear. The maximum slope of 0.3 pH units per hour has been obtained in solutions with glucose as a substrate. pH changes are associated with decomposition of the substrate by bacteria enzymes.

Various toxic substances can affect the behaviour of microorganisms and thus lead to a decrease of their enzymatic activity. Our preliminary experiments have shown that the presence of metal ions (Cu^{2+}) in the substrate solution has a pronounced effect on the kinetics of pH changes (Fig. 7). The possible application of this effect to the construction of a biosensor for toxic compound registration is obvious.

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